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AN EXPERIMENTAL STUDY OF THE DEVELOPMENT OF *SCHISTOSOMATIUM DOUTHITTI* (CORT, 1914) IN ITS INTERMEDIATE HOST[1]

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In spite of all the investigations that have been made on schistosome life cycles, surprisingly little is known of the development of the sporocyst stages and the germ cell cycle in this group. In a recent paper (Cort and Olivier, 1943a) results were reported of studies on natural infections of a member of this group, *Cercaria stagnicolae* Talbot, 1936, which have given some new information on these phases of development.

The present paper gives the results of studies on the sporocyst stages of *Schistosomatium douthitti* from experimental infections of snail intermediate hosts. All the observations were made on living material of mother and daughter sporocysts in infections of different ages (for methods, see Cort and Olivier, 1941).

The miracidia used in the experimental infections were obtained from eggs passed by laboratory mice which had been infected with cercariae of *S. douthitti* from natural infections in *Lymnaea stagnalis appressa* Say and *Lymnaea stagnalis perampla* Walker. In the summer of 1941 small juveniles of *Stagnicola emarginata angulata* (Sowerby) collected from a lake near the Biological Station were used for the experimental infections. This snail is obviously not a natural intermediate host for *S. douthitti,* since in the thousands that have been examined from the region only one infection has been found (Cort et al., 1937). During the summer of 1941 only mother sporocysts, not including the youngest stages or those that were fully developed, were obtained. We, therefore, returned to the study in the summer of 1942, using laboratory raised juveniles of a natural intermediate host, *Stagnicola palustris elodes* (Say). With an abundant supply both of the snails and infected mice, we were able to study the stages in the development of *S. douthitti* from very young mother sporocysts to mature daughters.

Time was not available for attempting single miracidium infections in the experimental snails, so that groups of them were infected in aquaria of various sizes simply by putting in fecal material containing the eggs or by pouring in miracidia that had hatched. In the summer of 1941 large numbers of eggs in fecal pellets were intro-

[1] A contribution from the University of Michigan Biological Station and the Department of Parasitology, School of Hygiene and Public Health, the Johns Hopkins University. This paper is one of a series on the embryology of digenetic trematodes in the intermediate hosts. The others have dealt with another schistosome (Cort and Olivier, 1943a), the strigeids (Cort and Olivier, 1941), and a plagiorchiid (Cort and Olivier, 1943b).

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duced over a period of about a week into an aquarium containing about 100 juveniles of *S. e. angulata* resulting in the infection of almost all the snails; in fact, multiple infections were the rule with some snails having as many as 5 or 6 mother sporocysts. In the summer of 1942 all the miracidia used in infecting the juveniles of *S. p. elodes* in a given aquarium were allowed to hatch and were introduced at one time, so that it was possible to know the age of the infections produced. In the experimental infections of this summer the majority of the snails were infected, but multiple infections appeared to be rare. The mother sporocysts were not difficult to find except in the very youngest stages, since as reported by Price (1931) they are localized in the snails, being found in the tissues close to the beginning of the esophagus near the cerebral ganglia.

DEVELOPMENT OF THE SPOROCYSTS OF *S. douthitti* IN EXPERIMENTALLY INFECTED SNAILS

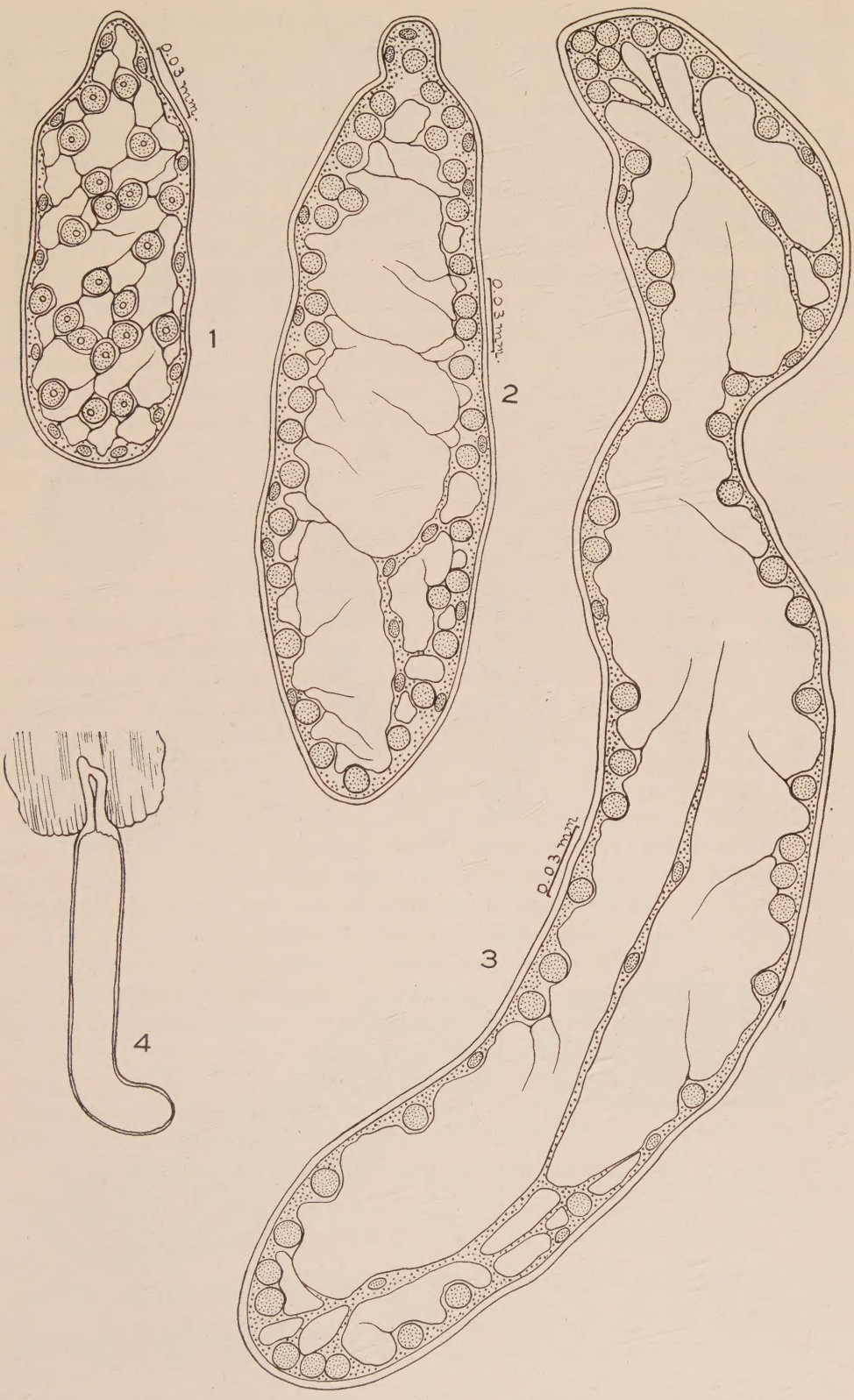
For convenience in description the development of *S. douthitti* in the intermediate host can be divided into three phases, viz., (1) the germ cell multiplication in the young mother sporocyst, (2) the embryonic development of the daughter sporocysts within the mother, and (3) the development of the daughter sporocysts in the tissues of the snail intermediate host.

Germ Cell Multiplication in the Young Mother Sporocysts

Five very young mother sporocysts were found in one group of *S. p. elodes* about 65 to 69 hours after exposure to the miracidia. They varied in length from about 0.12 to 0.20 mm and in width from about 0.05 to 0.08 mm. They had, therefore, grown only slightly since Price (l. c. p. 700) gave the average size of heat killed miracidia of *S. douthitti* as 0.131 by 0.060 mm. When these young mother sporocysts were freed from the snail's tissues, they were able to extend and contract the body with a slight wriggling movement, brief periods of rest being followed by periods of activity, which, however, did not result in any locomotion. The ciliated epithelium of the miracidium had been lost, and in two cases masses of material next to the sporocysts appeared to be remnants of this layer. Fig. 1 shows a drawing in optical section (camera lucida outline) of one of these very young mother sporocysts. All the structures that fill the anterior half of the miracidium (rudimentary digestive tract, penetration glands and nerve mass) have completely disappeared. The body wall is of uniform thickness and structure and the anterior end can be distinguished only by its greater mobility and attenuation. The inner layer of the body wall has a greyish granular appearance from numerous small globules in its cytoplasm and at intervals shows slight projections with the small oval nuclei. The germ cells are scattered throughout the body cavity either singly or in groups and are attached by cytoplasmic strands to the inside of the wall and to each other. Price (l. c. fig. 13) in an optical section of a miracidium of *S. douthitti* showed numerous germ cells (26 in all) with large nuclei, prominent nucleoli and a thin layer of cytoplasm with fiber-

FIGS. 1, 2, and 3. Very young mother sporocysts, in optical section, from experimental infections in laboratory raised juveniles of *S. p. elodes*. Fig. 1 about 65 hours and figs. 2 and 3 about 110 to 116 hours after infection. The constricted region in figure 3 near the anterior end was due to handling.

FIG. 4. Outline drawing of young mother sporocyst showing attachment to the snail's tissue by the attenuated anterior end. Size, 0.7 mm by 0.07 mm.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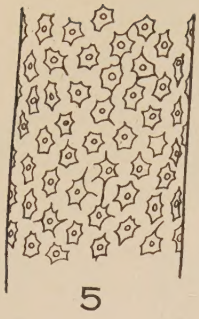

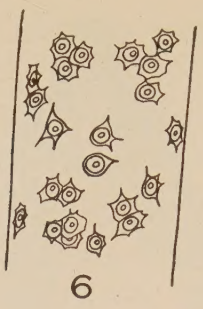

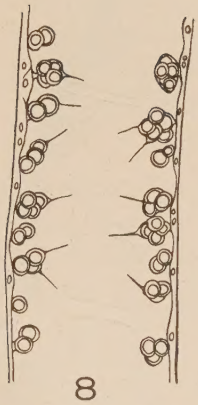

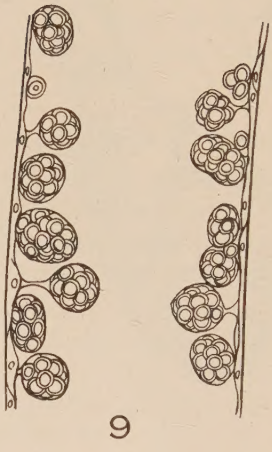

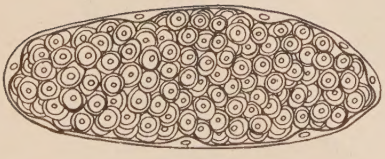

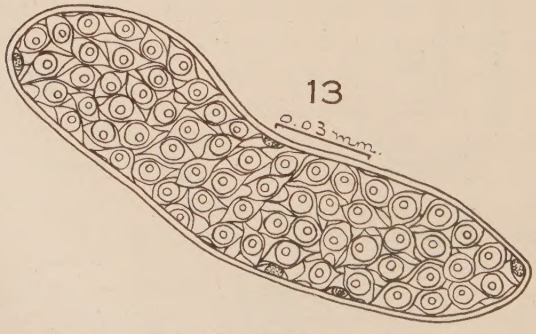

FIG. 5. Surface view of somewhat contracted young mother sporocyst showing the structure of the cells of the body wall. About 0.10 mm in width.

FIG. 6. View with focus just below wall of young mother sporocyst showing arrangement of undivided germ cells beneath the cellular layer of the wall. Scale about that of figure 5.

like processes connecting with the body wall. The germ cells of our youngest mother sporocysts had the same structure as those of the miracidium. Counts of these germ cells, which were probably under the actual number, ranged from about 30 to 40, suggesting that as yet there had been little, if any, increase in number.

Young mother sporocysts obtained from snails (*S. p. elodes*) 110 to 116 hours after exposure showed considerable further growth and development and a much greater variation in size. The germ cells had increased in number and were distributed along the inside of the body wall. This left a large central body cavity crossed by a few strands some of which contained nuclei. The nuclei of the germ cells showed no nucleoli as in the earlier stage, which suggested that they were in a phase of multiplication. Fig. 2 shows an optical section of one of the smallest of the mother sporocysts of this age, which has a size of about 0.30 mm by 0.09 mm. There is a distinct anterior tip enclosed in the host's tissue. The body wall appears thicker and somewhat more granular than in the earlier stage and the nuclei somewhat more scattered. The number of germ cells was estimated after repeated attempts to count them at somewhat less than 100. Fig. 3 shows the largest mother sporocyst found in the group of snails examined at 110 to 116 hours. The width is about the same as that of the specimen shown in Fig. 2 but the length is much greater (almost 0.6 mm). The inner layer of the body wall appears somewhat thinner and its nuclei are more scattered; the germ cells are more scattered even though they have increased in number; more than 150 were counted in this specimen. A few strands from the body wall, some containing nuclei, traverse the lumen of the enlarged body cavity.

After the stage shown in Fig. 3 there appears to be some further increase in the number of the germ cells of the young mother sporocysts. In most cases the germ cells begin to develop into embryos in mother sporocysts which are about a week old, although in the 1941 infections some at least two weeks old and 1.2 mm in length were found with no developing embryos. Fig. 4 shows the shape and method of attachment to the snail's tissue of a young mother sporocyst at this stage. In the largest mother sporocysts containing only germ cells the nucleoli of these cells can be clearly seen (Fig. 7) suggesting that multiplication had been completed. The body wall appears to be slightly thinner and more opaque than in the earlier stages; the nuclei of its inner layer are still easily seen in slight projections into the body cavity. They belong to cells of the body wall which appear in surface view as scattered stellate cells with numerous cytoplasmic processes as shown in Fig. 5 in a somewhat contracted specimen. Fig. 6, a view with the focus just below the body wall, shows the appearance of the germ cells from surface view. If the relation of

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FIG. 7. Optical section of a small part of a young mother sporocyst one to two weeks after infection, showing the relation of the germ cells to the body wall. Scale about that of figure 5.

FIG. 8. Optical section of somewhat older stage than figure 7, showing the relation of the early cleavage stages of the daughter sporocyst embryos to the body wall. About same scale as figure 5.

FIG. 9. Optical section showing the relation of the developing daughter sporocyst embryos (round "germ ball" stage) to the body wall. Width about 0.15 mm. Figures 5–9 were drawn from young daughter sporocysts from experimental infections in juveniles of *S. e. angulata*.

FIG. 10. Daughter sporocyst embryo in slightly later stage than those shown in figure 9. Diameter 0.050 mm.

FIGS. 11 and 12. Daughter sporocyst embryos showing early stages of elongation. Fig. 11, size 0.064 by 0.040 mm; Fig. 12, size 0.136 by 0.057 mm.

FIG. 13. Daughter sporocyst embryo slightly more advanced than in fig. 12, showing cytoplasmic projections from germ cells.

the germ cells in the miracidium and youngest mother sporocysts (Fig. 1) had not been known, it might easily have been thought that they developed in the inner layer of the sporocyst wall; for especially in contracted specimens they appear to form an inner layer of the wall (Fig. 7).

Development of Daughter Sporocyst Embryos Inside the Mother

Fig. 8 shows the early cleavage stages of the development of the embryos inside somewhat older mother sporocysts. At this stage, the cell groups are still attached closely to the inside of the body wall by protoplasmic strands, but they are more definitely separated from the wall than the germ cells of the earlier stages (cf. Figs. 7 and 8). In some sporocysts that were found with 2 to 8 cell cleavage stages, only a few single germ cells would be left; in others about half the germ cells would not have started development. Mother sporocysts with the germinal material at this stage varied in length from less than 1 mm to about 2.5 mm.

As development continued more and more of the mother sporocysts were found with embryos in the "germ ball" stage (Fig. 9). Such embryos are still attached to the inside of the wall, but stand out further into the body cavity. Along with the round "germ balls" a few germ cells and early cleavage stages are still present although most of the embryos are usually in about the same stage of development. During this stage of early development of the daughter sporocyst embryos, the young mother sporocysts continue to grow rapidly; also, their variation in size at any given age becomes greater. In one mother sporocyst of the 1941 series, 3.5 mm in length the embryos were almost all in this early round "germ ball" stage varying in diameter from 0.02 to 0.05 mm. In a 13-day old mother sporocyst of the 1942 series, which was 4.7 mm in length by 0.26 mm in width, the embryos were also in the round "germ ball" stage, the largest measuring 0.07 mm.

The embryo in the round "germ ball" stage (about 0.03 to 0.07 mm in diameter) consists of a mass of tightly packed cells surrounded by a thin membrane in which a few flattened nuclei can be seen (Fig. 10). From this outer membrane develops the body wall of the daughter sporocyst. The enclosed cells are typical germ cells like those of the mother sporocyst, with large nuclei, prominent nucleoli and a small amount of cytoplasm. They are so closely packed at this stage that it was impossible to determine whether they had cytoplasmic processes connecting them with the body wall or with each other.

After the embryos in the "germ ball" stage reach a diameter of about 0.05 to 0.07 mm they begin to elongate (Figs. 11 and 12). They are still attached to the inside of the body wall of the mother sporocyst and a few earlier stages and even single germ cells can still be found. In these elongate daughter sporocyst embryos, the wall appears thicker and the germ cells more numerous. Occasionally at this stage it was possible by careful observation to see cytoplasmic processes extending from the germ cells. Mother sporocysts containing embryos that had started to elongate had increased considerably in size. One, 13 days old and 8.4 mm in length, had most of its embryos showing elongation, the largest having a size of 0.11 by 0.09 mm. Another still larger mother (11.4 by 0.29 mm) found 15 days after infection contained still larger elongate embryos, the largest have a size of 0.12 by 0.09 mm. In these specimens the embryos were still attached to the wall; in fact, in one mother sporocyst, embryos that had begun to take the form of daughter sporocysts, which

were about the stage of Fig. 13 and almost 0.15 mm in length, were still attached to the wall.

At the stages of development just described it was possible to make some fairly accurate counts of the numbers of embryos present in individual mother sporocysts. One, 11.4 mm long, contained 172 embryos; in another 4.7 in length there were 206, and for a third 4.7 mm long the total count reached 420.

After the daughter sporocyst embryos reached the stage shown in Fig. 13 the germ cells usually appear slightly less crowded and cytoplasmic processes connecting them with the wall and with each other can usually be seen on careful examination. The nuclei of the body wall can be made out as projections into the body cavity. After this stage the daughter sporocyst embryos increase rapidly in length and begin to show activity. About this time, also, the connections of the embryos to the body wall of the mother sporocysts are broken down and they are found floating free in the body cavity. From this stage until the first daughters are ready to escape from the mother the growth in length of the daughter sporocysts proceeds rapidly. In an extraordinarily large mother sporocyst (26.6 mm in length) found 17 days after infection, almost all the daughter sporocyst embryos were distinctly elongate, varying from 0.11 mm by 0.08 mm to 0.45 by 0.08 mm. The largest showed considerable activity but had not yet reached the migrating stage.

A daughter sporocyst embryo which had almost completed its development and is approaching the migrating stage is shown in Fig. 14. Such daughter sporocysts are beginning to show considerable activity, indicating that the muscle layers of the wall are well developed. Some nuclei of the inner layer of the body wall can still be seen. The germ cells are usually not nearly so crowded as in earlier stages and their cytoplasmic projections can almost always be easily seen. There is, however, some variation in this respect, since in some mother sporocysts, daughters that appear almost ready to migrate have the germ cells still crowded, and their cytoplasmic processes can be seen only with difficulty.

By 20 days after infection most of the mothers contained some daughter sporocysts that appeared fully developed and ready to migrate. In one mother 20½ days old large numbers of active daughters were present that varied in length from 0.38 to 0.83 mm; in another of the same age there were large numbers of daughters that appeared ready to escape and only eight smaller embryos. In neither of the snails in which these two mothers were found were there any daughters outside the mothers.

The structure of a daughter sporocyst that appeared ready to migrate from the mother is shown in Fig. 15. Such daughters, the largest of which are slightly under 1 mm in length, are very active and can extend and contract the body greatly. The anterior end is distinctly pointed and covered with small backward-pointing spines. No attempt was made to work out the details of structure of the body wall. Evidently muscular layers are well developed. Occasional nuclei can still be made out in the inner layer of the wall which has become quite opaque from the numerous globules which it contains. In the specimen shown in Fig. 15 the germ cells are already loosely scattered in the body cavity and are held in place by their cytoplasmic processes. A few of them have already developed into embryos which are held in position by strands connecting with the wall. This represents a precocious development of the germ cells, since in the majority of fully developed daughters observed

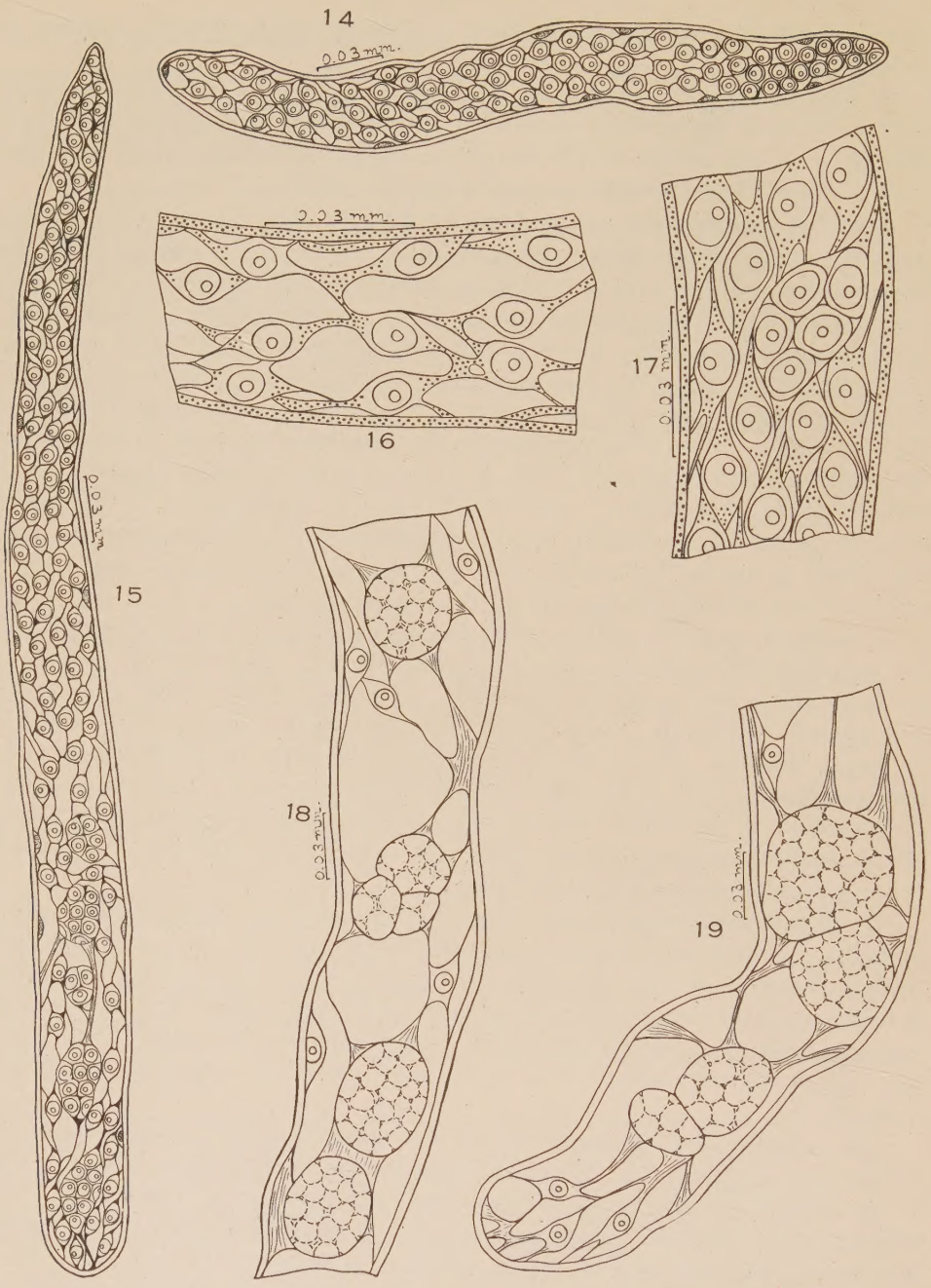

FIG. 14. Daughter sporocyst embryo, just before migrating stage, from a 17-day-old mother sporocyst.

FIG. 15. Daughter sporocyst which has completed its development and appears ready to migrate from a 20½-day-old mother.

FIG. 16. Optical section of a small part of a migrating daughter sporocyst 1.7 mm long from the digestive gland of a snail 21½ days after infection. In this case none of the germ cells had started to develop although they were separated by spaces on account of the rapid growth of the sporocyst.

inside of the mothers only germ cells were present, which were usually closer together than in the sporocyst shown in Fig. 15. In fact, in a few cases daughters outside the mothers, which were considerably longer than any found still inside the mother sporocysts, contained no developing embryos. One such daughter, which was 2.06 mm long, contained about 238 germ cells and no developing embryos. On the other hand, precocious development of the embryos may be even greater than shown in Fig. 15. In a 17-day old mother sporocyst in which none of the daughters appeared ready to migrate and the largest were at about the stage shown in Fig. 14 a few 4 to 6 cell embryos were found among the germ cells. Also, in another mother 20½ days old the largest daughters contained a considerable number of embryos; in one 0.83 mm long there were 11 fairly large germ balls, the largest of which had already grown to a size of 0.061 by 0.029 mm, and a number of smaller ones.

It seemed of interest to determine how many germ cells are produced during the phase of multiplication in the daughter sporocysts. Sixteen counts of the germ cells of daughter sporocysts still within the mother gave numbers from 127 to 208 with an average of 170. These counts were difficult to make and our impression is that they are considerably short of the actual numbers. In fact, counts in three daughter sporocysts in the migrating stage which had left the mother gave 238, 227 and 236 germ cells respectively and the third had also 5 small germ balls.

Mature and old mother sporocysts, which in some cases persist long after the first daughters have escaped, vary greatly in length (from about 10 to 26 mm). Their body wall is quite thin and opaque from the increase of the minute globules in its inner layer. Such mother sporocysts when still in the snail look like inflated, elongate sausage shaped balloons, and the germinal material even before any of the daughters have escaped fills only a part of the body cavity. Their anterior ends are attenuated and birth pores and canals, as described for the mother sporocysts of *C. stagnicolae,* can be clearly seen (Cort and Olivier, 1943). Apparently the daughter sporocysts escape only from the birth pores of the mother, since empty sporocysts still intact have been not infrequently found.

Time of Escape of Daughter Sporocysts and Persistence of Mothers

As already indicated, at any given stage in the development of a mother sporocyst most of the daughter sporocyst embryos appeared to be in about the same stage of development. Thus as noted above in one mother 20½ days old, from which no daughters had yet escaped, there were numerous active daughters 0.38 to 0.83 mm in length and only eight embryos in earlier stages. This rather synchronous development of the daughter sporocyst embryos inside of the mother usually results in the escape of most of the daughters over a comparatively short period of time. The youngest infection in which we actually found daughter sporocysts outside the mother was 21½ days old. In this case 36 daughters, which were active and which appeared ready to migrate, were counted inside the mother; no earlier embryonic stages were present. The large numbers of daughters outside the mother (84 were

FIG. 17. Optical section of a small part of a migrating daughter sporocyst from the digestive gland of a snail 22½ days after infection. Although some of the germ cells had already started to develop into cercarial embryos the germinal material was still rather tightly packed in the body cavity.

FIGS. 18 and 19. Optical sections of small portions of young daughter sporocysts from the digestive gland of snails showing development and arrangement of the germinal material.

isolated and counted) were still in the migrating stage, although the largest were about twice the length of those still inside the mother sporocyst. While this suggested that the first of these daughters probably escaped earlier than 21 days after infection, still the actual lag in development of those still within the mother was not very great. In another infection only 23 days old the mother sporocyst was entirely empty and the daughters had completed migration and had penetrated into the digestive gland. This case also indicates that the escape from the mother took place over a short period of time although it seems probable that the first sporocysts had escaped before 21 days. In another infection 27½ days old the mother was also entirely empty and the daughters had developed so far and were so interlaced in the tissues of the snail's digestive gland that it was difficult to remove any complete.

Usually, however, while most of the daughters will escape at about the same time, a few that have lagged behind the others will be found inside the mother. This is well illustrated by two infections in which the mother sporocysts 41 days after infection were still in good condition and contained a few active daughters and earlier embryos; in both cases the daughters in the tissues of the snails were almost mature and contained cercariae that appeared ready to escape.

A few of the infected snails were examined at much longer periods after infection. In 10 cases mother sporocysts containing a few daughters, most of which were active, and a few germ balls were found in snails 61 and 65 days after infection. In fact mother sporocysts with a few active daughters still in them were found in snails examined 71, 88, 126, 144 and 154 days after infection. In the group of 10 snails examined 154 days after infection, however, mother sporocysts were found in only two but in both these cases active daughters were present. Finally, in 10 snails examined 167 days after infection no mothers were found. The snails which were examined at the longer intervals after infection had been subjected to low temperatures for considerable periods of time which had delayed the development of the sporocysts. It seems clear, however, that mothers containing a few daughters can persist for a considerable time. Whether daughters that have remained in the mothers for such long periods after the others have escaped will ever migrate and develop cannot be determined, but in many old mother sporocysts, daughter sporocysts and embryos that had obviously degenerated were not infrequently found.

The general impression we have gained from the examination of large numbers of infections is that in most cases most of the daughter sporocysts of *S. douthitti* develop almost synchronously and escape from the mothers at about the same time. This produces infections in which most of the daughter sporocysts are of about the same age. In fact, in only a very few cases have we ever found immature daughters along with mature daughters that were producing cercariae.

Development of Daughter Sporocysts After Leaving the Mother

Migrating daughter sporocysts are located on the organs between the foot and digestive gland of the snails. They are very active and mobile and can extend and contract their bodies greatly. They increase considerably in size even before migration is over. In the 21½ day-old infection mentioned above, the daughters within the mother which were active and appeared ready to escape measured 0.42 to 0.74 mm, and those outside the mother which were still in the migrating stage varied from 0.78 to 2.22 mm. Due to this rapid growth the germinal material in the larger

of the migrating daughters becomes much less crowded. Also, more of the germ cells have started embryonic development, although as noted above, there is considerable variation in these respects in different infections. Fig. 16 shows a small section of a migrating daughter sporocyst 1.7 mm in length in which none of the germ cells had started development, which illustrates how the rapid growth during migration spreads the germ cells apart. On the other hand, Fig. 17 shows a section of a migrating daughter sporocyst, 1.6 mm in length, from another infection, in which some of the germ cells had developed into embryos, but in which the germinal material was more crowded. Still further spreading apart of the germinal material is shown in Fig. 18. This is drawn from a migrating daughter sporocyst which contained 236 germ cells, 5 developing embryos and one germ-mass. It is the youngest daughter sporocyst in which a germ-mass was found. This infection was started August 11, 1942, and was much retarded in development, since at 37 days after infection the mother still contained 36 daughters, and those outside the mother were very immature.

After migration most of the daughter sporocysts penetrate into the digestive gland of the snail; only a few penetrate into the tissues among the organs in front of this gland. After penetrating into the tissues they become completely immobile. Most of the germ cells soon start to develop into embryos which increase rapidly in size. The sporocysts evidently continue to increase in size, since even with the rapid development of the germinal material it fills only a part of their body cavities. The body wall of the sporocysts becomes more opaque and appears to be thinner than in the earlier stages. The wall is in very close contact with the snail's tissue and it is almost impossible to free sporocysts unbroken; in fact, the largest daughter sporocyst that was ever freed unbroken from a snail's tissue was only 2.5 mm in length, and was only slightly advanced in development beyond the migrating stage. Fig. 19 shows the posterior end of a daughter sporocyst soon after establishment in the digestive gland. As shown in this drawing the germ cells are beginning to take a position close to the inside of the wall and the largest embryos have a diameter almost equal to that of the body cavity of the sporocyst. At this stage, also, all the embryos and germ cells are still connected by strands to the wall. In slightly later stages the few germ cells that remain undeveloped are always close to the wall and more and more of the lumen is filled with the embryos. In somewhat older daughter sporocysts no undeveloped germ cells are found and the embryos are free in the body cavity, having lost their connections with the wall.

At about the stage when the largest cercarial embryos become elongate and reach a length of 0.10 to 0.15 mm, the daughter sporocysts begin to show inflated sections containing the embryonic material separated by empty constricted sections. Fig. 20 is an outline drawing of a part of a sporocyst showing the general shape at this stage. In such sporocysts undeveloped germ cells were never found. The inflated sections contain cercarial embryos in various stages of development and occasional germ-masses (Figs. 21 and 22). All attachment of the embryos to the wall has been lost. In slightly older sporocysts, in which some of the cercarial embryos are beginning to show a division into body and tail, the inflated sections become broader and are more nearly filled by the developing embryos (Figs. 23 and 24). The wall of the daughter sporocysts at this stage is quite opaque, and so thin and in such close contact with the snail's tissue that only small sections of the individual sporocysts can usually be freed.

As shown in Figs. 21, 22, 23 and 24 germ-masses similar to those found in sporocysts of *C. stagnicolae* (Cort and Olivier, 1943) may be present along with the cercarial embryos in the inflated sections of the daughter sporocysts. They are not at all common and it is unusual to find as many as three in a single inflated area. Ex-

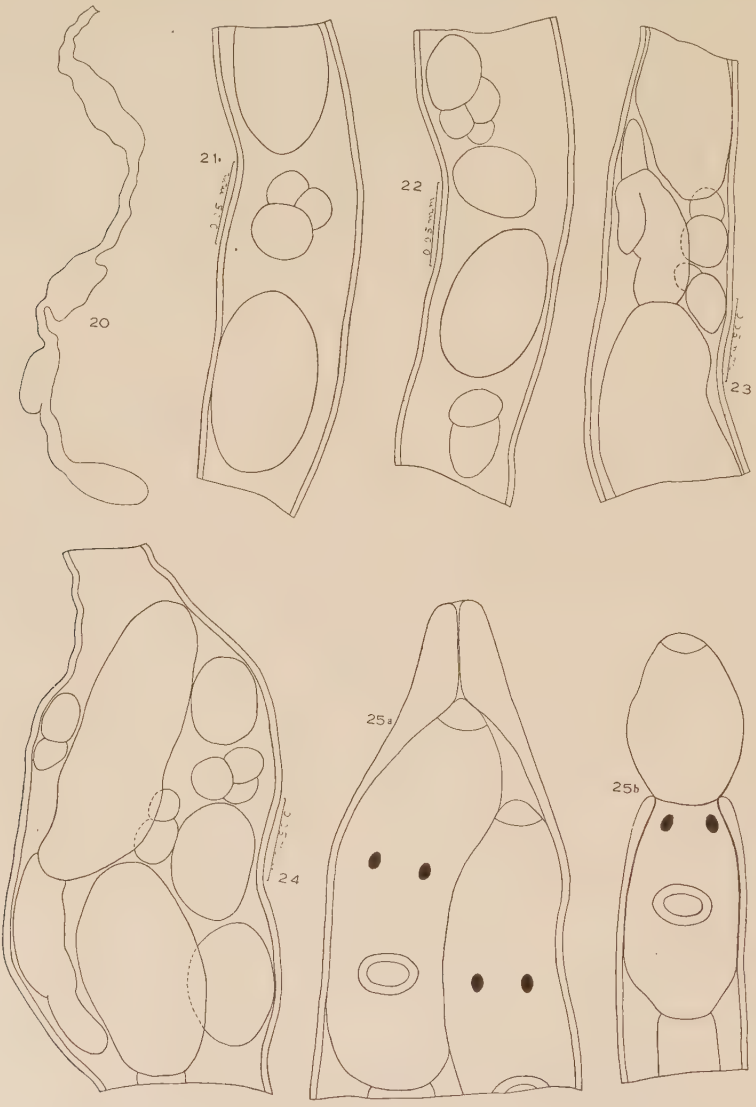

Fig. 20. Outline drawing of an incomplete daughter sporocyst from snail's tissue showing relations of the inflated sections.

Figs. 21, 22, 23 and 24. Outline sketches showing relation of cercarial embryos and germ-masses in small sections of daughter sporocysts about the stage of figure 20.

Fig. 25a and b. Sketches showing anterior end of daughter sporocysts containing fully developed cercariae.

cept in a very few cases germ-masses were not found at stages earlier than those shown in Figs. 22 and 23; and they were very rarely present along with mature active cercariae. In some infections we were unable to find any germ-masses at all and in others numerous sections of sporocysts would be examined before any were found.

In only a few of the infections examined did we find them at all regularly and then only in part of the sporocysts and in about the numbers shown in Figs. 21 to 24. Of course, the germ-masses are difficult to find and are obscured by the opacity of the sporocyst wall, so that we could not always be sure that failure to find them was due to their absence. The germ-masses found in our experimental infections as shown in Figs. 21 to 24 seem to be almost entirely composed of multicellular components, which vary in number from two to five (usually 3 and 4). In several natural infections of sporocysts of *S. douthitti* studied at about this same stage of development small numbers of germ-masses were also found. In a few of these, unicellular components were present.

When the cercariae in an inflated section of a daughter sporocyst approach maturity they tend to crowd it to its full capacity. Often such sections appear to contain only almost fully developed cercariae. In mature infections from which cercariae are escaping the daughter sporocysts permeate the whole tissue of the digestive gland, appearing to make up at least half its bulk. The digestive gland appears to be badly injured especially in infections in which a large proportion of the sporocysts are empty and degenerate.

If the body of a snail containing a mature infection of *S. douthitti* is removed from the shell, terminal sections of the sporocysts with attenuated anterior tips and birth pores (Fig. 25a and b) will be seen extending slightly from the surface of the digestive gland. No escape of cercariae from these birth pores was ever seen until the examinations were made in the evening. To observe the escape, six snails with natural infections of *S. douthitti* were isolated in bottles and the water changed in the late afternoon. They were watched during the evening and the cercariae began to escape from them about 9:30 P.M. (Eastern War Time). At 9:45 P.M. the body of one of these snails was removed from the shell, and a number of cercariae were soon seen to emerge from the birth pores of the anterior tips of the sporocysts which projected from the surface of the digestive gland. This observation confirms the earlier findings of Cort and Talbot (1936) on the time of escape of the cercariae of *S. douthitti* from the snail host.

DISCUSSION

The miracidium of *S. douthitti* like those of other schistosomes contains a considerable number of germ cells in its body cavity, indicating a multiplication of the cells of the germinal line beginning at an early stage of its development. After penetration into the snail intermediate host the miracidium metamorphoses into an oval sac with these germ cells scattered throughout its body cavity (Fig. 1). As this sac grows rapidly in length the germ cells start to multiply again and come to lie close to the inside of the body wall (Figs. 2 and 3). While it would be difficult to prove, it seems probable that the phase of germ cell multiplication in the very young mother sporocyst is completed before any of them start development. In mother sporocysts about a week old the germ cells start to develop into embryos which remain attached to the wall until they have reached considerable size. All the germ cells of the mother sporocysts develop directly into daughter sporocyst embryos since no structures that had the slightest resemblance to the germ-masses that were so common in the mother sporocysts of *C. stagnicolae* (Cort and Olivier, 1943) were ever found. By the time these embryos have reached the round "germ

ball" stage they consist of a mass of germ cells enclosed in a thin cellular membrane. As these embryos elongate the wall becomes thicker and the enclosed germ cells increase in number. They can be seen to have cytoplasmic processes connecting them with the inside of the body wall and with each other. With further increase in length these embryos develop into elongate motile daughter sporocysts with a well differentiated body wall, which contain a large number of germ cells. Either just before or just after the daughters escape from the birth pore of the mother their germ cells start to develop into embryos. After the daughter sporocysts leave the mother they migrate along the organs of the snail and penetrate into the tissues chiefly in the digestive gland. After penetration they become immobile and later appear as much larger irregular elongate sacs with inflated sections containing the developing embryos and sometimes a few germ-masses separated by narrow empty sections. The attenutated anterior ends of the daughter sporocysts with birth pores extend slightly from the surface of the digestive gland of the snail.

In the experimental infections started in *S. p. elodes* about the first of August, 1942, the first daughter sporocysts escaped from the mother about three weeks after infection and the first cercariae began to escape from the daughters at about 6 weeks. It, therefore, took about two weeks for the germ cells of the mother to develop into migrating daughters and about three weeks for the germ cells of the daughters to become active cercariae. With warmer temperatures the time of development would undoubtedly be considerably shorter. Also, in the experimental infections started later in August development was much slowed up by cooler weather.

Multiplication of individuals in the sporocyst stages of *S. douthitti* is brought about almost entirely by two phases of multiplication of the cells of the germinal line. One of these starts in the early embryo of the miracidium and is completed several days after the mother sporocyst has started its development in the intermediate host. This results in the production of a considerable number of daughter sporocysts (from about 200 to over 400) inside the mother, and each germ cell of the mother appears to develop directly into a daughter. The second phase of germ cell multiplication must start very early in the development of the daughter sporocyst embryos because in germ balls less than 0.05 mm in diameter the soma is represented only by a thin membrane in which only a few nuclei can be seen, and a number of germ cells are already present. As the outer membrane differentiates to form the wall of the daughter sporocysts the germ cells increase in number to about 200 before they start embryonic development.

It is evident that all but a few of these germ cells in the daughter sporocyst embryos develop directly into cercarial embryos. A very few of them, however, must develop into the germ masses which are found in small numbers in some of the developing daughter sporocysts. These, we suggest, represent a very brief phase of polyembryony in which a single germ cell divides and its divisions start development without separating. When these muticellular components of the germ-mass reach a certain size they break apart and each develops into a cercaria. Thus in the daughter sporocysts of *S. douthitti* it would appear that each of the few germ cells that develop into germ-masses finally produces several cercariae. Since these germ-masses are entirely absent in the mothers and so few in number in the daughters, it is evident that polyembryony of germ-masses in this species plays only a very minor part in the multiplication of individuals. A conservative estimate would be

that in *S. douthitti* from the fertilized ovum that produces the miracidium 40,000 to 60,000 cercariae are produced, almost entirely by the two phases of direct multiplication of germ cells. The multiplication of individuals in the germ cell cycle in this species, therefore, consists of four phases, viz., (1) the production of large numbers of fertilized ova by the adults, (2) multiplication of cells of the germinal line in the miracidium-mother sporocyst stage, (3) multiplication of cells of the germinal line in the daughter sporocyst stage, and (4) a very brief and irregular phase of polyembryony in the daughter sporocyst stage.

Since in an earlier paper (Cort and Olivier, 1943) on the embryology of the sporocysts of *C. stagnicolae* the meagre information in the literature on the development of the sporocysts of the schistosomes was discussed, it will be sufficient to point out here the differences between *C. stagnicolae* and *S. douthitti.* In general appearance and structure the mother sporocysts of these two species are very similar although their location in the intermediate host is entirely different. Also, in the early stages of development the germinal material in the mother sporocysts of *C. stagnicolae* does not line the wall but is suspended by strands in the lumen. A fundamental difference between these two species is the presence in the mother sporocysts of *C. stagnicolae* of a considerable number of germ-masses which are completely absent in this stage of *S. douthitti.* So polyembryony in the mother sporocysts of *C. stagnicolae* appears to be an important method of increasing the number of daughter sporocysts, while it is entirely absent at this stage in *S. douthitti.* There is, therefore, a greater lag in the production of daughter sporocysts in the former species and a tendency for their development and escape to spread over a longer period of time.

The structure of the stages of development of the daughter sporocysts of the two species inside the mother is very similar. In these stages of *C. stagnicolae* the multiplication of germ cells seems to last longer, since when the daughters in this species escape from the mother the germ cells in their body cavity are very crowded and none was ever seen that had started development. In fact, in this species we never saw cytoplasmic projections from the germ cells of daughters while still within the mother, although judging from later stages they must be present. It can be suggested that the extent of crowding of the germ cells in the developing daughter sporocysts depends on the time relations of germ cell multiplication to growth in size. In *S. douthitti* there is considerable variation in this respect. Apparently in some cases the multiplication of the germ cells inside the daughter sporocyst embryos continues until the daughters are ready to escape. This results in a condition like that of *C. stagnicolae* in which the germ cells are rather crowded and none has started embryonic development. In a few cases in *S. douthitti,* however, it appears that the germ cell multiplication in the daughters inside the mother is completed earlier, resulting in the spreading apart of the germ cells and some precocious development of embryos in the daughter sporocysts before they escape from the mother.

Another difference between these two species is the presence in *C. stagnicolae* of large numbers of germ-masses in the early stages of the development of the daughter sporocysts outside the mother. In fact, these structures are so common that the suggestion was made that possibly all the germ cells go through this stage before producing cercarial embryos. Whether this is true or not, it is evident that polyembryony of germ-masses in the daughter sporocysts of *C. stagnicolae* is a very

important factor in the multiplication of individuals while in *S. douthitti* it seems to be a very minor factor, and perhaps only occasionally present.

While actual counts are not available we have a clear impression that in *C. stagnicolae* cercarial production is very much greater than in *S. douthitti.* Also, cercarial production in the former species appears to extend over a considerably longer period than in the latter. The extent of polyembryony of germ-masses in the former species and its almost complete suppression in the latter would account for these differences. In fact, it seems possible that *S. douthitti* is exceptional among shristosome species in the small number of cercariae produced and in the comparatively short period in which they escape from the snail host. We certainly have the impression that cercarial production is also very much greater in the other two schistosome cercariae of the Douglas Lake region, *C. elvae* and *C. physellae.* It is of special interest in this connection that in a limited series of observations we were able to make on immature mother sporocysts of *C. elvae* in natural infections we found germ-masses with about the same frequency as in *C. stagnicolae.* We know of no records of the presence of germ-masses in the sporocysts of other schistosome species. It is of interest, however, that studies of single miracidium infections in *Schistosoma mansoni* (Faust and Hoffman, 1934) indicated a considerably greater cercarial production over a very much longer period than for *S. douthitti.* In one such infection 210,000 cercariae were actually counted and the snail was still discharging more than 2,500 cercariae per day when the counts were discontinued. It can be suggested, therefore, at least as a working hypothesis, that the presence of a considerable number of germ-masses with a significant polyembryony is typical of the schistosomes, and that the situation in *S. douthitti* is unusual for the group. However this may be, it seems evident that in the germ cell cycle of the schistosomes the direct multiplication of the cells of the germinal line is very important in the production of large numbers of Cercariae, and that polyembryony of germ-masses which in the strigeids accounts for most of the multiplication, is relatively of less significance.

SUMMARY

In the summers of 1941 and 1942 studies were made on the development of the mother and daughter sporocysts of *Schistosomatium douthitti* in experimentally infected snails. In the youngest mother sporocysts only germ cells were present, which increased in numbers to about 200. These germ cells developed directly into daughter sporocysts, which escaped from the birth pore of the mother after about three weeks. Germ cells localized in the body cavities of the daughter sporocysts increase in numbers to about 200 when the daughters are ready to escape from the mothers. Most of these develop directly into cercarial embryos, the first of which mature in about six weeks. A few germ-masses are present in the daughter sporocysts after they are established in the snails' tissues, suggesting that a brief phase of multiplication by polyembryony is also involved. It was estimated that from 40,000 to 60,000 cercariae are produced from one fertilized ovum, almost entirely by direct multiplication of the cells of the germinal line in the mother and daughter sporocysts. The almost complete elimination of multiplication by germ-masses in the sporocysts of this species appears to have limited cercariae production, as compared with species like *C. stagnicolae* and *C. elvae* where these structures are numerous in both the mother and daughter sporocysts. It is evident that direct multiplication of

the cells of the germinal line in the mother and daughter sporocysts plays a much greater role in the multiplication of individuals in the schistosomes than in the strigeids, since in this group multiplication is chiefly by polyembryony of germ-masses.

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THE EFFECT OF CONCURRENT INFECTION WITH *NIPPOSTRONGYLUS* ON THE DEVELOPMENT OF *HYMENOLEPIS* IN MICE[1]

JOHN E. LARSH, JR.[2] AND ALAN W. DONALDSON[3]

A few observations have been made but little or no specific work has been done to show what effect the presence of one species of parasite has on the development of a second. Brumpt (1933) noticed that the resistance of one mouse to reinfection with *Hymenolepis nana* var. *fraterna* was broken down by a secondary infection with *Strongyloides*. Apparently there are no reported observations on the mutual effect of two different parasites following simultaneous entry into a host. It was to obtain such information that the present study was undertaken, since the nematode, *Nippostrongylus muris,* and the cestode, *H. nana* var. *fraterna,* were both available in the laboratory at the same time.

METHODS

Quantitative doses of *Hymenolepis* eggs isolated from host feces which had been stored 48–72 hours were administered to mice by stomach tube, and 93-hour cysticercoid counts were made after preparing the intestine free from mucus (Larsh, 1942). Infective *Nippostrongylus* larvae were isolated from 10–20 day old cultures, washed by centrifugation in normal saline, and counts made by a modification of Scott's (1928) dilution count. Known numbers of larvae were injected subcutaneously into mice in the abdominal wall just medial to the flank.

EXPERIMENTAL DATA

Three preliminary experiments were performed in which a total of 24 mice, 2–3 months old, were used. Half of these were infected subcutaneously with 1000–1500 *Nippostrongylus* larvae per mouse just prior to receiving 1200–1350 eggs of *Hymenolepis* per mouse. The other 12 mice which were used as controls received only the tapeworm eggs. In the first experiment, the four experimental mice had an average percentage development of cysticercoids of 1.3, and the controls 2.2. The second experiment showed a striking difference between the groups as the four experimentals averaged 1.6, and the controls 4.5. The third experiment was more nearly comparable to the first in that the experimentals averaged 1.2, and the controls 2.7. No record was kept of the *Nippostrongylus* infections, since, at this time, the larvae are still in the migratory stage. These results were striking enough to suggest an interesting relationship worthy of further study, so that two other experiments were performed in which larger numbers of animals were involved and the doses of larvae and eggs kept constant.

In the first experiment, twenty mice two months old were selected. Ten of these were each infected subcutaneously with 2000 larvae of *Nippostrongylus* and within

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an hour were each given 4200 *Hymenolepis* eggs. The ten controls were given a corresponding number of eggs of *Hymenolepis* from the same culture. In the second experiment, another twenty mice, two months old, were used and the ten experimental animals each received 2400 larvae of *Nippostrongylus*. Later, along with the controls, they were each given 2150 eggs of *Hymenolepis*. The results of the two experiments are shown in Table 1.

An examination of the table shows that the two experiments are markedly similar and that the number of cysticercoids of *Hymenolepis* in the controls was about twice the number observed in the experimental group. In the first experiment, the controls had a percentage development of cysticercoids of *Hymenolepis* of 6.0, compared with 3.7 in the mice infected with both parasites, and, in the second experiment, the controls averaged 6.6, the experimentals 3.0 per cent. These results agree, therefore, with those of the three preliminary experiments, so that in all a total of 32 experimental and 32 control mice were observed.

TABLE 1.—*Showing the percentage development of cysticercoids in mice infected with both* Hymenolepis *and* Nippostrongylus *and in mice infected only with* Hymenolepis

Exper. no.	No. of mice	Infecting egg dose per mouse	Ninety-three hour cysticercoids		
			Average no.	Standard deviation of the individual measurements	Percentage development
A. Mice infected simultaneously with *Hymenolepis* and *Nippostrongylus*					
1	10	4200	156.3	19.2	3.7
2	10	2150	64.6	18.0	3.0
B. Control mice infected only with *Hymenolepis*					
1	10	4200	253.5	32.3	6.0
2	10	2150	142.4	20.9	6.6

The data of the last two experiments, Table 1, were analyzed statistically. In the first experiment, the observed difference between the percentage development of the cysticercoids in the experimental and control groups was 7.7, and in the second experiment 8.4, times the standard deviation. Grouped together for analysis, the two experiments show an even more pronounced significance, since the observed difference between the percentage development of the total animals of the two groups was 10.9 times the standard deviation. Hence it is extremely unlikely that the differences shown in the table could have occurred by chance.

The results of these experiments indicate that the infections with *Nippostrongylus muris* in some way or other reduced the numbers of the cysticercoids of *H. nana* var. *fraterna* that were able to develop.

SUMMARY

Young white mice infected with *Nippostrongylus muris* just prior to infection with *H. nana* var. *fraterna* exhibited a marked resistance to the tapeworm and harbored only one-half the number of cysticercoids observed in controls of the same

age infected only with the cestode. No suggestion is offered to explain this unusual phenomenon, but further work is planned to study the mechanism involved.

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COMPARATIVE STUDIES ON A MOUSE STRAIN OF *HYMENOLEPIS NANA* VAR. *FRATERNA*, IN DIFFERENT SPECIES AND VARIETIES OF MICE[1]

JOHN E. LARSH, JR.[2]

As a result of the many studies on *Hymenolepis nana* var. *fraterna* in white mice, much information has been presented on the relations between this parasite and host. Few data have been published, however, on any of these relations in other species and varieties of mice. Hunninen (1935) compared the percentage development of the parasite in white mice with that in deer mice (*Peromyscus maniculatus*) and other rodents, but he had opportunity to test only a small number of the wild mice. Therefore, when the writer was presented with several mating pairs of deer mice and house mice (*Mus musculus*), it seemed worth while to make further tests with these hosts. In addition, a series of other comparisons were made between white and dilute brown laboratory mice.

EXPERIMENTAL DATA

The first comparison between the albino and wild mice was in the number and location of cysticercoids after initial infections (Table 1). The first point of interest in table 1 is the presence in all three groups of about the same percentage development, the range for the white mice, house mice, and deer mice being respectively

TABLE 1.—*A comparison of the percentage development and location of cysticercoids of* H. nana *var.* fraterna *in white mice,* M. musculus *and* P. maniculatus

No. of mice	Age of mice (days)	No. eggs per mouse	Ninety-three hour cysticercoids				
			Average no.	Standard deviation of the individual measurements	Per cent in first half of intestine	Per cent in second half of intestine	Percentage development
A. White Laboratory Mice							
5	51	2700	98	6.8	85	15	3.5
4	25	2400	71.5	8.5	76	24	2.9
4	30	2000	63	6.4	94	6	3.0
4	60	2400	93.7	6.5	88	12	3.8
B. *M. musculus*							
5	51	2700	92.4	10.4	81	19	3.3
4	25	2400	67.2	8.9	84	16	2.7
4	30	2000	41.7	9.2	91	9	2.0
4	180	2400	87.2	9.5	92	8	3.6
C. *P. maniculatus*							
5	51	2700	85.6	5.1	34	66	3.1
4	25	2400	89.7	8.6	26	74	3.9
4	30	2000	90	14.6	25	75	4.4
4	180	2400	91	14.7	39	61	3.7

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2.9–3.8, 2.0–3.6, and 3.1–4.4. The second point of interest is the lack of resistance exhibited by the old (180 days) house mice and deer mice which had about the same percentage development as the younger mice. This is in contrast to the marked resistance shown by old white mice of this age (Shorb, 1933, Hunninen, 1935). Unfortunately, the three hosts could not be compared directly because no white mice 180 days old were available at the time of the experiment. A third point of interest is the location of the cysticercoids in mice of the three groups. The number found in the first half of the small intestine of the white mice and house mice was 76 per cent or more of the total number which agrees with Hunninen's (l.c.) figures for white mice. However, in the deer mice the reverse was true and only 25 to 39 per cent were observed in the corresponding region while most of the others were located in the posterior one-fourth of the small intestine. This unusual location of the cysticercoids was found in all the deer mice including those 180 days old.

The next experiments were performed to make a comparison between the different kinds of mice in the number and size of adult worms found after test infections; in all cases, the mice were autopsied 11 days following infection. The number of worms developing in the white and house mice was about the same, but very few were found in the deer mice (Table 2). The range in percentage development of

TABLE 2.—*A comparison of the number of 11-day worms of* H. nana *var.* fraterna *in white mice,* M. musculus *and* P. maniculatus

No. mice	Age of mice (months)	Egg dose per mouse	11-day adult worms		
			Average no.	Standard deviation of the individual measurements	Percentage development
A. White laboratory mice					
5	2½	2000	37.2	11.9	1.9
3	2½	2500	58.6	8.6	2.3
3	2½	2500	62.6	15.5	2.5
B. *M. musculus*					
5	2½	2000	30.8	10.0	1.5
3	3½	2500	63.3	5.0	2.5
3	5½	2500	60.0	14.7	2.4
C. *P. maniculatus*					
5	2½	2000	1.00	1.260	0.05
3	3½	2500	0.66	0.027	0.03
3	5½	2500	0.00	0.000	0.00

the worms in the white mice was 1.9–2.5, in the house mice 1.5–2.5, and in the deer mice 0.03–0.05. Another point brought out is that the old house mice (5½ months) had about the same percentage development of the adult worms as the younger mice. Those tested when 2½ and 3½ months old had a percentage development of 1.5 and 2.5, respectively, compared with 2.4 at 5½ months. A third interesting point is the differences in the size of the worms from the three hosts. Those recovered from the house mice were always larger than those from the white mice while the few worms found in the deer mice were very much smaller. In the first series when 2000 eggs were fed, the average size of the 186 worms from the five white mice was 39 mm. (range: 30–51), that of the 154 worms from the house mice was 49 mm. (range: 38–67), and the average size of the 5 worms from the deer mice was 3 mm.

(range: 2–8). The relationship in size was similar in the other two experiments, but, as stated above, direct comparisons were not possible because white mice of the same age as the wild mice were not available when needed. It was found that size is not necessarily associated with host age as the worms recovered from the old house mice (5½ months) were about the same size as those from the younger mice of this species.

In the comparisons between the white and dilute brown[3] mice, the first point to settle was whether the two hosts differ in their natural susceptibility to *H. nana* var. *fraterna*. This determination was based on the results of five experiments in which a comparison was made of the number of 93-hour cysticercoids present after test infections of stored eggs (Larsh, 1943b). In every case, the white mice, from 52–82 days old, harbored considerably more parasites than the brown mice of the same age given similar infections from the same egg culture (Table 3). The white

TABLE 3.—*Showing the percentage development of cysticercoids in white and dilute brown mice infected with different egg doses of* H. nana *var.* fraterna

Exper. no.	No. of mice	Age of mice (days)	Egg dose per mouse	Ninety-three hour cysticercoids		
				Average no.	Standard deviation of the individual measurements	Percentage development
A. White Laboratory Mice						
1	8	52	900	36.5	7.0	4.0
2	4	75	1200	54.0	8.3	4.5
3	3	82	1500	61.6	5.3	4.0
4	5	75	2000	101.8	9.5	5.4
5	5	75	2000	112.4	7.5	5.6
B. Brown Laboratory Mice						
1	8	52	900	5.3	3.9	0.6
2	4	75	1200	23.5	8.6	1.9
3	3	82	1500	37.0	4.0	2.4
4	5	75	2000	54.2	13.5	2.7
5	5	75	2000	47.0	9.7	2.4

mice showed a range in percentage development of cysticercoids from 4.0 (first and third experiments) to 5.6 (fifth experiment), while the range for the brown mice was from 0.6 in the first to 2.7 in the fourth experiment.

The next tests were designed to obtain data on the adult stage of the parasite, all the mice being killed eleven days after infection when the number and size of the worms were recorded. In the three experiments performed, each of the 18 white mice harbored more than twice as many worms as the brown mice (Table 4). The range in percentage development was from 2.3 to 3.2 in the white mice, and from 1.0 to 1.9 in the brown mice. The worms recovered from the two hosts did not vary greatly in size. Those from the white mice after an infection of 3500 eggs, while showing the usual variations (10–61 mm.), averaged 31.5 mm. in length, as compared with 26.2 mm. in the brown mice where the range in size was 6–43 mm.

The last tests were performed to determine the prepatent and patent periods of the worms in the two hosts. Twelve mice, 2½ months old, of each variety were infected with 1500 eggs per mouse. After the tenth day, all the fecal pellets collected over a 12-hour period each day were examined for eggs by the Willis salt-flotation method. Five white mice began passing eggs on the fifteenth day, four on

[3] The C57 strain bred in the Jackson Memorial Laboratory, Bar Harbor, Maine.

TABLE 4.—*Showing the percentage development of adult* H. nana *var.* fraterna *in 2½-month-old white and dilute brown mice infected with different egg doses*

Exper. no.	No. of mice	Egg dose per mouse	11-day adult worms		
			Average no.	Standard deviation of the individual measurements	Percentage development
A. White Laboratory Mice					
1	5	2000	52.4	13.6	2.6
2	8	2000	45.0	12.0	2.3
3	5	3500	113.2	12.6	3.2
B. Brown Laboratory Mice					
1	5	2000	20.8	7.6	1.0
2	8	2000	23.1	8.7	1.1
3	5	3500	67.2	13.1	1.9

the seventeenth, two on the eighteenth, and one on the twenty-second day. Five of the brown mice began passing eggs on the eleventh day, six on the twelfth, and one on the thirteenth day. The length of the patent period was also recorded by using these same twelve mice of each variety and later by adding an additional six in each group which were infected with the same number of eggs, i.e., 1500. Daily fecal examinations were continued until each animal had been negative for seven days, then the absence of worms was verified by autopsy. The patent period of the worms in the white mice ranged from 20 to 48 days, and in the brown mice from 16 to 30 days.

DISCUSSION

After test infections of the same size, similar numbers of cysticercoids of *H. nana* var. *fraterna* were observed in white mice, deer mice, (*Peromyscus maniculatus*) and house mice, (*Mus musculus*), but in later experiments, the deer mice harbored only a few adult worms while the other two hosts had equally large numbers. These results, in general, are in harmony with those of Hunninen (1935) who tested white mice, deer mice, rats and guinea pigs and found a somewhat similar development of cysticercoids in all hosts; but, in other experiments, he observed very few adults in any but the white mice. He pointed out that the experiment showed a specificity of the adults and a lack of specificity of the cysticercoids. This same relationship was demonstrated again in the above series of experiments. It is interesting that the old house mice (180 days) harbored about the same number of cysticercoids and adults as the young mice of this species because old white mice are resistant to infection. If more extensive study proves that old house mice are as susceptible in all cases, then it may offer some support to Sandground's (1929) hypothesis that age immunity is merely a type of natural immunity. This author contends that age immunity is shown typically by a host against only abnormal, newly acquired, or imperfectly adjusted species of parasites. Thus the reason old white mice exhibit strong resistance to *Hymenolepis* may be due to slight physiological changes produced in establishing the strain from the wild *Mus musculus*. Regardless of the view taken, *Mus musculus* appears to be a better host for this tapeworm than white mice heretofore referred to as the "normal" host.

It is clear from the above data that certain differences exist between the white and the brown laboratory mouse in their relationships with *H. nana* var. *fraterna*.

The smaller numbers of cysticercoids and adult worms in the brown mice and the shorter patent period would seem to suggest that they are less favorable for the development of the parasite than white mice. It is, therefore, rather surprising that the parasite has a shorter prepatent period in the brown mice, because this indicates more rapid development to sexual maturity. The difference in intestinal length in these two varieties of mice might be suggested as a possible explanation for the difference in the number of worms that develop in them. A series of measurements for mice 75–82 days old showed that the intestinal length of the brown mice averaged 35.5, while in the white mice it was 45 cms. In an earlier paper (Larsh, 1943a), experimental evidence was presented in support of the view that the smaller intestinal size of young white mice is an important factor in their lower susceptibility to *Hymenolepis* than mice 2–3 months old. No test was made of this relationship in the above experiments, but the suggestion is presented as one possibility for the reduced susceptibility of the brown mice because the size of their intestine is similar to that of the young white mice studied previously. However, whatever explanation is presented to account for the results of the study must give consideration to the very apparent factor of genetic constitution which differs in the two hosts.

Ackert and his coworkers (1935) were the first to give evidence of different degrees of host susceptibility to helminthic infections between breeds of the same species. They studied chickens infected with the nematode, *Ascaridia lineata,* and found that the heavy breeds, e.g., Rhode Island Reds, were the least susceptible while the lighter breeds, e.g., White Leghorns, were the most susceptible.

SUMMARY

The host relations of *H. nana* var. *fraterna* were tested in different species and varieties of mice. After the same test infections, white mice, house mice (*Mus musculus*), and deer mice (*Peromyscus maniculatus*) showed about the same number of cysticercoids, but in later tests the deer mice harbored only a few adult worms whereas similar large numbers were recovered from the other two hosts. *Mus musculus* appears to be the best host for this parasite, because the worms grow to a larger size than in other hosts tested, and old mice of this species are as susceptible as younger members. Comparisons between white and dilute brown mice showed that after the same test infections, the brown mice harbored fewer cysticercoids and adults than did white mice of the same age. The adult worms in the two hosts grew to about the same size, but the prepatent and patent periods were shorter in the brown mice.

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EFFECT OF LONG ULTRAVIOLET AND NEAR VISIBLE RADIATION ON THE EGGS OF THE NEMATODES *ENTEROBIUS VERMICULARIS* AND *ASCARIS LUMBRICOIDES*

MYRNA F. JONES AND ALEXANDER HOLLAENDER

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The effect on nematode eggs of ultraviolet radiation shorter than 3000A has been reported in a number of publications. The present authors (Hollaender, Jones, and Jacobs, 1940) have reported on the effect on eggs of *Enterobius vermicularis* of radiation with wavelengths between 2180 and 3000A, the greatest sensitivity of the eggs to radiation being noted at wavelengths below 2400A. The effects of sunlight on nematode eggs, especially eggs of the various species of *Ascaris* also have been under consideration for a number of years. Under the conditions of many of these experiments with sunlight there existed very little chance that the eggs received ultraviolet shorter than 3000A in sufficient quantity to produce toxic effects, but in spite of the practical absence of the shorter ultraviolet, definite inactivating effects have been observed.

By recently available experimental arrangements, it has become possible to produce high intensity radiation in the range of 3500A to 4900A, i.e., in the long ultraviolet and near visible range. Effects of these high intensities are comparable to those produced by sunlight in which the shorter ultraviolet has been excluded. This report is concerned with the results observed in using radiation of the 3500 to 4900A range on eggs of *Enterobius vermicularis* and *Ascaris lumbricoides*.

MATERIAL AND METHODS

Source of radiation.—As a light source there was used a water-cooled high-pressure quartz capillary mercury vapor lamp of the Daniels-Heidt type (150 to 250 volts and 2 to 4.5 amperes). Radiation from the capillary lamp was concentrated by means of a quartz condensing lens and passed through a liter round-bottom glass flask filled with a dilute solution of copper sulphate. The light then passed two Corning glass filters #585—3 mm and #738—2 mm. The beam was deflected by means of a right angle reflection prism to a platform on which was placed a uranium glass plate. The light beam covered an area of 1.5 by 5 cm clearly visible on the fluorescent plate. Usually two or three dishes of eggs were placed in the beam, and their location interchanged every 10 to 20 minutes, depending on the time of exposure. The arrangement of the equipment is shown in Fig. 1.

The intensity of the beam was measured by means of a standardized thermopile-galvanometer arrangement. The intensities obtained varied between 10,000 and 300,000 ergs per cm^2 per second. Spectrograms taken of the radiation coming to the ova covered the range between 3500 and 4900A. The most intense lines were the 3650A set followed by the 4358 group and finally by the 4046 set and some minor lines. Only very little radiation longer than 4358 was present. Practically all infra red radiation was absorbed by the $CuSO_4$ solution and no measurable quantity of ultraviolet shorter than 3500A could be detected.

Eggs of Enterobius vermicularis.—Eggs of *E. vermicularis* were irradiated in the infective stage. As in other experimental work with this parasite by the present authors (1940), eggs were obtained from female worms which had been collected while migrating; the eggs were incubated at 37° C for about 7 hours for development of the vermiform infective-stage embryo, then stored in a refrigerator until used. With one exception, eggs were used within a week after incubation. Only eggs from one worm were used in each experiment. During irradiation the eggs either were floating on water in small dishes, or were kept dry, adhering somewhat to the bottoms of the dishes. With few exceptions each sample consisted of 150 to 200 eggs. The criterion of effect of irradiation was the subsequent ability of living larvae to hatch in artificial digestive juice; this four-hour hatching test was begun immediately following irradiation.

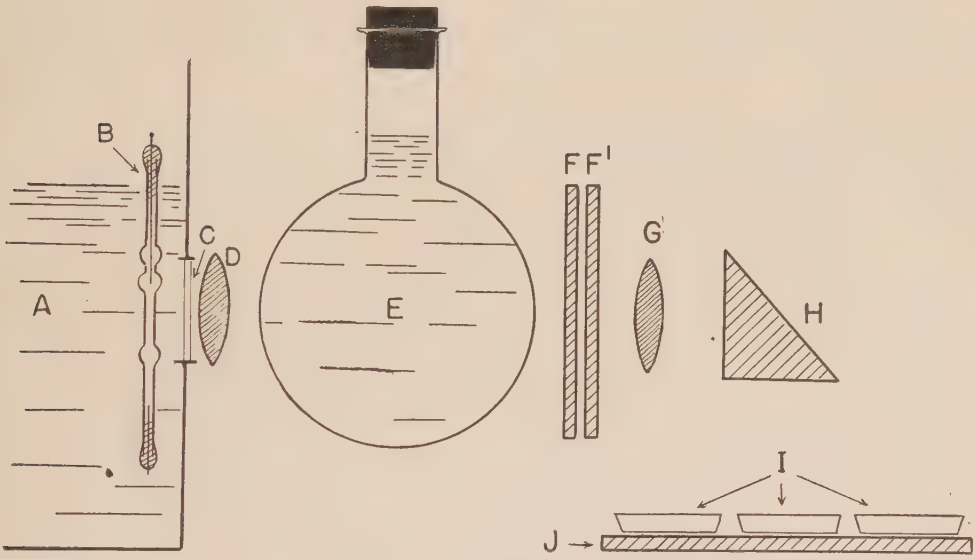

FIG. 1. Apparatus. A, Water bath for radiation source. B, Capillary mercury vapor lamp. C. Quartz window of lamp tank. D, Quartz condensing lens. E, Pyrex glass bottle with $CuSO_4$ water solution. F and F', Corning glass filters #738 and #585. G, Quartz condensing lens. H, Right angle reflection prism. I, Dishes containing eggs. J, Fluorescence plate.

To obtain information regarding temperature factors, in certain experiments the dishes of eggs were kept on a warm plate, and in others were surrounded by cracked ice. However, eggs were not subjected to conditions of temperature or humidity which would, *per se,* noticeably decrease their viability during the time of exposure to radiation, which did not exceed 4 hours.

Eggs of Ascaris lumbricoides.—Eggs were obtained from worms which had been passed by patients after treatment. They were stored in shallow dishes of water in a refrigerator until used. Certain of the eggs were incubated at 30° C to obtain late-segmentation stages and embryonated forms. Most of the eggs, however, were in the one-cell stage when irradiated. During irradiation, the eggs were in shallow dishes, either dry or in distilled water. Effort was made to have eggs in single layers so that all eggs were similarly exposed. In some experiments, samples of eggs for testing were taken from a dish at intervals; in other experiments, eggs were in separate dishes which were removed at appropriate times. Some of the embry-

onated eggs were on small glass coverslips which could be mounted on a slide to facilitate observation after irradiation.

The criterion of effect of irradiation on eggs in the one-cell and the late-segmentation stages was the subsequent ability to complete embryonation at 30° C. Embryonated eggs were checked for activity of the vermiform embryo when warmed to approximately 37° C. The last type of test was less satisfactory quantitatively and resulting data are not considered as reliable as those for the tests based on development. However, after irradiation with the higher energy values, visible damage to the embryo was evident.

In earlier experiments it was not possible to hold eggs longer than 15 days after irradiation, but in later tests eggs were kept as long as 28 or 30 days for final observation.

All eggs were irradiated at room temperature which varied from 24° to 29° C; no data were obtained concerning possible temperature factors.

RESULTS

E. vermicularis.—Data of experiments with *E. vermicularis* are summarized in Table 1. Considerable inconsistency is noted between experiments at room temperatures (between 20° and 30° C) in which the maximum exposure was to radi-

TABLE 1.—*Summary of data on effect of irradiation with 3500A to 4900A on eggs of* E. vermicularis

Exp. No.	Date	Age of eggs (days)	Eggs dry or wet	Temp. °C	Relative humidity (per cent)	Minimum exposure $\times 10^7$ ergs per cm^2	Survival	Maximum exposure $\times 10^7$ ergs per cm^2	Survival
1	8/13	5.5	dry	30–31.5	65	50.2	+	200.	0
2	8/14	6.5	wet	30	..	7.5	+	24.5	+
3	8/15	7.5	wet	28–29	..	18.8	+	24.5	+[1]
		7.5	dry	28–29	50	18.8	+	24.5	0
4	10/2	5.5	wet	21.5–24.5	..	...	..	6.3	+
		5.5	dry	21.5–24.5	48	...	..	6.3	+
5	10/8	3.5	wet	27–29	..	15.7	+	23.6	+
		3.5	dry	27–29	50	15.7	+	23.6	+
6	10/9	4.5	wet	28	..	89.	+	145.	0
		4.5	dry	28	50	...	..	145.	0
7	10/10	5.5	wet	25		17.	+	26.8	+[1]
		5.5	wet	25		13.1	+	26.8	+[1]
		5.5	wet	37–39		13.1	+	26.8	+[1]
8	12/11	3.5	wet	23–25		33.	+	134.	+[1]
		3.5	wet	35–38		67.	+	134.	0
9	12/19	(a) 1.5[3]	wet	37		40.	+	100.	0
10	12/20	(a) 2.5	wet	8–13		35.	+	89.	+[2]
		(b) 2.5	wet	8–13		35.	+	125.5	+[2]
11	12/23	(a) 5.5	wet	8–15		74.7	+	199.2	+[2]
		(b) 5.5	wet	8–15		87.2	+	199.2	+[2]
12		(a) 5.5	wet	23–28		74.7	+	149.6	0
		(b) 5.5	wet	23–28		49.8	+	149.6	0

[1] Less than 10% hatching.
[2] Damaged larvae.
[3] a and b represent eggs from 2 different worms.

ation less than 50×10^7 ergs per cm^2. In some tests there was considerable killing effect by low energy values of about 25×10^7 ergs per cm^2; however, whether these results were correlated with less resistant material, unmeasured fluctuation in light source, or other factors, it was apparent that such radiation could not be relied upon to kill eggs under all circumstances. It was only with much greater energy values that consistent killing at room temperatures was observed.

Figure 2 presents data from experiments designed to test the importance of a temperature factor correlated with the effect of radiation. In each test, eggs of the

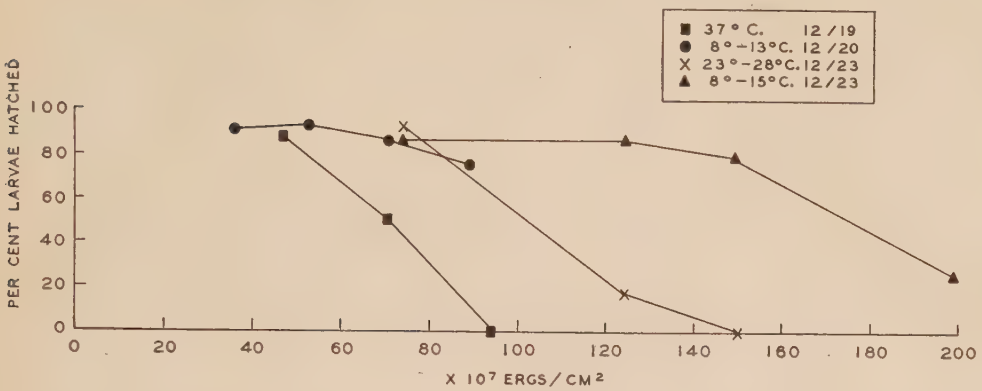

FIG. 2. Effect of 3500 to 4900A radiation on eggs of *Enterobius vermicularis* exposed at three different temperature ranges.

same lot were used, with the same lamps. In some cases it was possible to irradiate eggs at different temperatures on the same day; if not, exposures were made at the higher, possibly more effective temperature on the first day. Consistent results were observed. At temperatures between 35° and 38° C the effect of irradiation was most pronounced; at temperatures between 23° and 28° C killing was less rapid. At temperatures below 20° C, that is between 8° and 15° C, many eggs survived exposures up to 199×10^7 ergs per cm². It was noted, however, that although many larvae were able to hatch after such exposures, some showed evidence of damage, such as a brownish color or sluggish movements. It is probable that continued irradiation could have been effective, even at these low temperatures.

Comparisons of effect of radiation on dry eggs and eggs on water indicate that there was little difference in the tests with approximately 75 per cent of wet eggs hatching after irradiation. In the few tests which indicated some difference, dry eggs were apparently the less resistant.

Ascaris lumbricoides.—Results of the first series of experiments with eggs in the one-cell stage were checked on the basis of embryonation at 12 to 15 days after irradiation. Exposure of less than 30×10^7 ergs per cm² produced slight or no effect. Radiation of 60 and 80×10^7 ergs per cm² resulted in embryonation of only

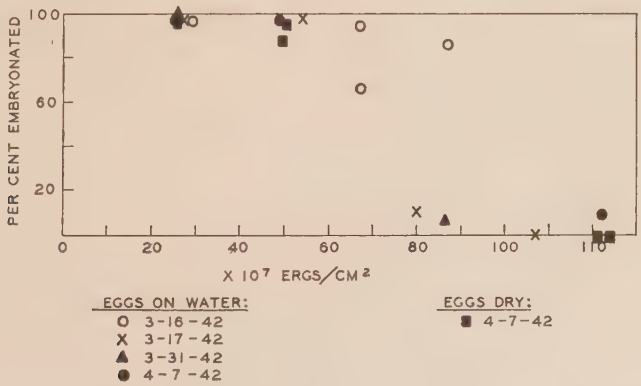

FIG. 3. Effect of 3500 to 4900A radiation on one-celled eggs of *Ascaris lumbricoides.* Final check on development after 4 weeks at 30° C.

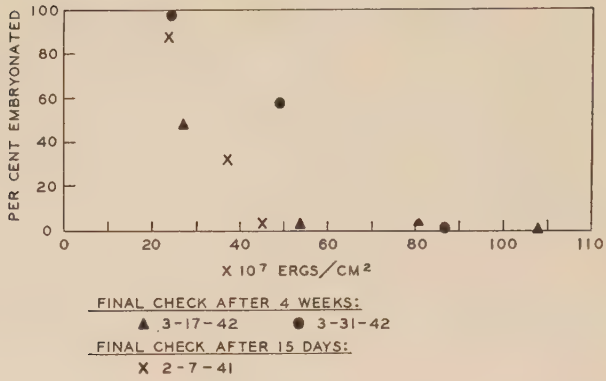

FIG. 4. Effect of 3500 to 4900A radiation on eggs of *Ascaris lumbricoides* in late-segmentation stages.

10 per cent or less of the eggs, whereas within the same time 96 to 99 per cent of the unexposed, control eggs embryonated.

Results of the second series of experiments with one-celled eggs, which were incubated longer, that is for approximately 4 weeks after irradiation, are presented in Fig. 3. Little effect was produced by radiation with energy values up to 55×10^7 ergs per cm², and in one test 86 per cent of the eggs embryonated after exposure to 87×10^7 ergs per cm² although development was delayed; of all the other eggs exposed to radiation of approximately that value or to radiation of greater energy values only 10 per cent or less embryonated. No eggs in 2 of 3 samples exposed to 123×10^7 ergs per cm² and only a few eggs in a clump near the edge of the dish of the third sample were able to embryonate; unembryonated eggs of these 3 samples remained in the one-cell stage; 98 and 99 per cent of two control samples embryonated.

In the limited tests made, eggs in late-segmentation stages exhibited no greater resistance to irradiation with this wavelength range than did the one-celled forms. Additional testing of this point is indicated since increased development of organisms has been correlated with increased resistance to irradiation with short ultraviolet. Results with three different lots of eggs are presented in Figure 4. Data for embryonated eggs are similar to those for eggs in late segmentation stages. However, as noted above, the criterion of effect of irradiation, that is the activity

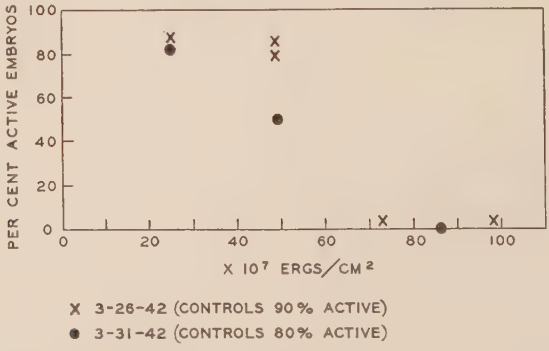

FIG. 5. Effect of 3500 to 4900A radiation on embryonated eggs of *Ascaris lumbricoides*.

of the warmed vermiform embryo is much less satisfactory and the observed results are not exactly comparable with those based on development.

Within individual experiments, in spite of some variability, increased, continued irradiation with sufficient energy effectively reduced the percentage of survival of eggs in any stage of development.

In the few tests in which the resistance of eggs in water and that of dry eggs were compared, there were no marked differences but such differences as there were suggest that the dry eggs may be somewhat less resistant.

A comparison of the rates of embryonation after various exposures is presented in Fig. 6. A delay in development of some irradiated eggs was observed. Such a delay was also apparent in the first few days during early cell division. In one

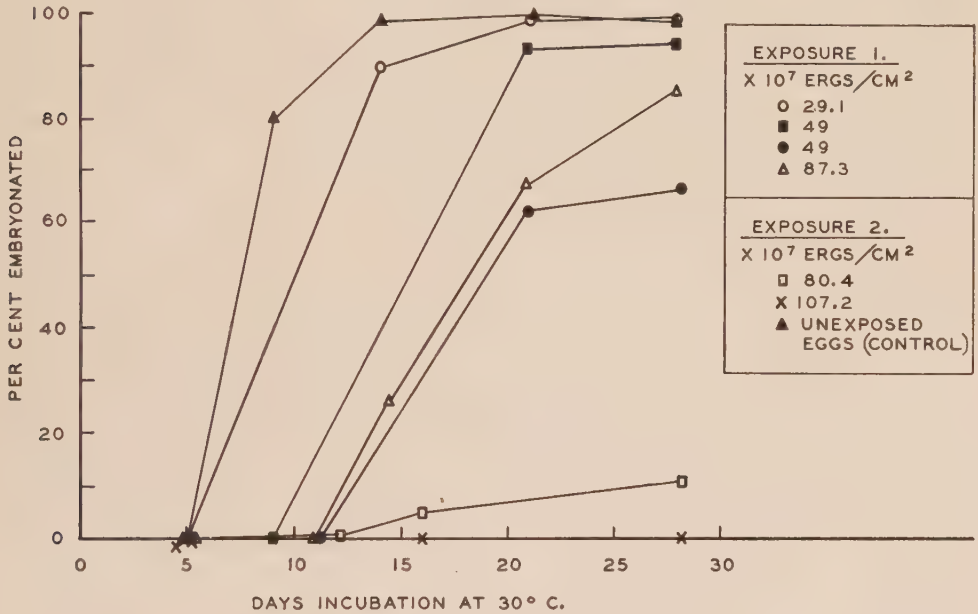

FIG. 6. Rate of embryonation of eggs of *Ascaris lumbricoides* after various exposures to 3500 to 4900A radiation.

experiment, on the fifth day of incubation, control eggs were predominately in 8 and 16-cell stages, eggs exposed to 29.1×10^7 ergs per cm² radiation were predominately in 2, 4, and 8-cell stages; eggs exposed to 87.3×10^7 ergs per cm² were chiefly undivided, only 1.3 per cent being in 2 and 4-cell stages. In another experiment, on the sixth day of incubation, eggs exposed to 26.8×10^7 ergs per cm² were predominately in the 8-cell stage; no eggs which had been exposed to 80.4×10^7 ergs per cm² had completed the first cell division, although ultimately 10 per cent became embryonated.

DISCUSSION

The energy necessary to produce a damaging effect with radiation in this 3500 to 4900A range is very great, especially if compared to the ultraviolet radiation at wavelengths shorter than 3000A. For eggs of *E. vermicularis,* the energy necessary for lethal action at the wavelengths above 3500A is approximately 1000 times that necessary at wavelengths below 3000A.

In experiments by Wright and McAlister (1934) on eggs of *Toxocara canis* and *Toxascaris leonina,* no apparent effects were noted after exposure to approximately 27×10^6 ergs per cm^2. These results are comparable to those reported here for eggs of *A. lumbricoides* since consistent, striking effects were observed only after exposures to 123×10^7 ergs per cm^2, energy which is roughly 50 times that used by the above authors.

The region of the spectrum which was produced experimentally is quite intense in sunlight although the distribution of wavelengths (within the range) is not the same. In sunlight there would be much higher intensity at the longer wavelengths in contrast to this filtered mercury radiation which has the highest intensity around 3650A. Sunlight would contain considerable quantity of infrared radiation and, since higher temperatures appear to enhance the effect of the region used experimentally, sunlight might be expected to be more damaging than the mercury radiation used in this investigation. Sunlight, even though lacking in short ultraviolet, and not necessarily combined with high tropical temperature, should be expected to be lethal for nematode eggs after adequate time of exposure.

The use of sunlight, whenever practicable, would appear to be of value as a factor in the destruction of pinworm eggs and especially so if correlated with high temperature and low humidity.

The results observed in these investigations are to some extent comparable with observations made in Puerto Rico by Spindler (1940) on eggs of the pig ascarid exposed to tropical sunlight at temperatures of approximately 30° to 35° C.

Studies by Hollaender, Cole and Brackett (1939) and Hollaender (1940) on other organisms exposed to the light range used in these experiments have indicated that it might be primarily the respiratory system which is affected. No specific studies with the present material were made as to the method of effect, but nothing in the results of the present study appear to contradict such an interpretation. The delayed development of some irradiated ascarid eggs might be compared to the delayed development which has been observed when they are under conditions of reduced oxygen consumption.

SUMMARY

Eggs of the nematodes *Enterobius vermicularis* and *Ascaris lumbricoides* were subjected to long ultraviolet and near visible radiation in the 3500 to 4900A range, exclusive of short ultraviolet and infrared radiation.

Lethal effects were observed after radiation with sufficient energy. The energy necessary in this wavelength range is of different order of magnitude than the energy necessary for comparable effects at wavelengths below 3000A.

A lowered resistance of the eggs of *E. vermicularis* to radiation at higher temperature was observed.

A possible relation of these findings to the observed effects of sunlight is discussed briefly.

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RESEARCH NOTES

TRICHOMONAS VAGINALIS IN THE FLUID OF THE VAGINA

Since *T. vaginalis* has usually been studied in a liquid medium (as a rule in some salt solution enriched with serum) we were interested in knowing how it looked and how it behaved in the vagina. With the cooperation of Dr. Weinstein of the Department of Obstetrics and Gynecology of the Medical School of the University of Pennsylvania we procured a small amount of frothy material from the vagina of a negro woman. The specimen was taken with a speculum and a drop of it was examined under the microscope. When it was found to contain many trichomonads it was transferred to a test tube by means of a pipette. The tube was put on a brass inclined plane made for examining tissue cultures in test tubes and with the high dry objective we were able to study the flagellates. The thick creamy white mass consisting of squamous cells, debris and frothy fluid spread out as a long white streak on the wall of the test tube. The trichomonads were swimming freely among the cells and the debris. They had the usual pear shape with a protruding axostyle. The anterior flagella and undulating membrane were active. They varied in size, some being much larger than others. They pushed the cells about just as they do in a salt solution. Clusters of them were seen attached to debris. They were quite plastic and able to push in between the body cells. In fact, in the undiluted material taken from the vagina the trichomonads looked and behaved in all respects like those seen in salt solutions. In other words there was sufficient fluid present in the vagina for them to maintain an active free-swimming existence.—M. J. Hogue, *University of Pennsylvania Medical School, Philadelphia, Pa.*

A NEW METHOD FOR QUANTITATIVE ESTIMATION OF MICROFILARIAE IN BLOOD SAMPLES

In counting microfilariae most investigators have employed thick blood films or material recovered from laked and centrifuged blood samples. With the dehemoglobinization technique there is a danger of washing off some of the microfilariae while with the centrifugation method there is a possibility of losing microfilariae in transferring the material. It is believed that the procedure described below obviates such disadvantages.

Twenty cubic millimeters of blood are drawn up into a pipette such as is employed for the hemoglobin estimation by the acid hematin technique. After wiping the tip of the pipette with cotton, the volume of blood is expelled into the chamber of the Sedgwick-Rafter counting cell. This cell was designed for enumerating organisms in water and consists of a slide with a depression 0.1 cm in depth and 2×5 cm in area, thus capable of holding 1 cc of fluid. One cc of 0.1 N hydrochloric acid is added, the suspension stirred with a dissecting needle, and a cover slip applied without leaving an air bubble in the chamber. The microfilariae settle rapidly to the bottom of the chamber and little focusing is thus required. With the aid of a mechanical stage, the entire area of the chamber is examined with the use of a 15 or 25 mm objective.

The method permits the examination of quantities of blood up to 0.1 cc, obviates the possibility of loss of microfilariae in the test sample, and requires only a single piece of equipment. The only disadvantage encountered is that objectives providing magnification higher than 8 mm cannot be used because of the thickness of the preparation.—Frederick J. Brady and Alfred H. Lawton, *Division of Zoology, National Institute of Health, U. S. Public Health Service.*

THE INCIDENCE OF *EMBADOMONAS INTESTINALIS* WENYON AND O'CONNOR IN FOOD HANDLERS AND DIARRHEIC PATIENTS OF MENTAL HOSPITALS

Embadomonas intestinalis Wenyon and O'Connor, is considered a rare parasite. Most statistics and surveys do not mention it at all. Contrary to its recorded scarcity, this flagellate was found in 15, or 1.45 per cent of the 1029 examined inmates of State Hospitals of the Greater Chicago area. Of the patients 796 were checked bacteriologically and parasitologically during a survey of food handlers in hospital kitchens and on hospital wards; 233 inmates were examined for diarrheic conditions.

Embadomonas intestinalis was found in the stools of 9 or 1.1 per cent of the 796 apparently healthy food handlers. In 6 cases no other parasite was found. The flagellate was accompanied, however, once by *Endolimax nana* and twice by *Endamoeba coli*. Furthermore, the parasite was present in 6 or 2.6 per cent of the 233 diarrheic stool specimens collected from patients showing clinical symptoms of salmonellosis, bacillary or amoebic dysentery. In one case, *Shigella sonnei* was isolated from the feces containing *E. intestinalis*. In a second instance, *Giardia lamblia* and *Endamoeba coli* were found together with *E. intestinalis*. In the third and fourth cases, *Enda-*

moeba histolytica accompanied *E. intestinalis*. The stools of the fifth and sixth patients did not reveal other parasites than *E. intestinalis* and no bacterium considered as pathogenic could be cultivated from the same feces.

The frequent findings of *E. intestinalis* in State Hospital inmates can be explained by the greater incidence of intestinal microorganisms of doubtful or proven pathogenicity in chronically institutionalized mental patients, due to the less hygienic habits and housing of such persons on many a State Hospital ward.

The present observations do not warrant ascribing a pathogenic role to *E. intestinalis* but they serve as a stimulus for further investigation of the significance of flagellates in food handlers and diarrheic patients.—Viola Mae Young and Oscar Felsenfeld, *Department of Public Welfare of Illinois and Department of Pathology and Bacteriology of the Chicago Medical School, Chicago 12, Illinois.*

OBSERVATIONS ON A SPOROZOAN PARASITE OF THE EELPOUT, *ZOARCES ANGUILLARIS*, WITH AN EVALUATION OF CANDLING METHODS FOR ITS DETECTION

During the recent national campaign encouraging the increased production and utilization of relatively little-used species of marine fishes to help increase the food supply, the eelpout (*Zoarces anguillaris*), heretofore landed only occasionally, was being landed in fair abundance at several New England ports, and prepared for market by filleting. In the course of the increased handling of this fish a protozoan parasite was discovered inhabiting its flesh. A sample of the parasite sent to Dr. Ross F. Nigrelli of the New York Zoological Society was identified by him as belonging to the class Sporozoa, order Haplosporidia, and genus *Ichthyosporidium*.

Table 1.—*Results of various methods of candling the same 100 eelpout fillets (Fish from Cape Cod Bay)*

Procedure	Infections in lots of 25 fillets each				Total infected	
	I	II	III	IV	No.	%
Skin on						
No candling—cut side only	1	0	1	1	3	3.0
Candling, rapid—cut side only	1	1	2	2	6	6.0
Candling, detailed—cut side only	1	3	3	5	12	12.0
Skin off						
No candling—both sides	1	0	1	1	3	3.0
Candling, rapid—cut side only	3	2	2	5	12	12.0
Candling, detailed—cut side only	4	3	5	6	18	18.0
Candling, rapid—both sides	5	4	6	6	21	21.0
Candling, detailed—both sides	11	9	7	7	34	34.0

The parasite, in its trophozoite stage, lies within and parallel to the long axis of the muscle fibers, producing a hyaline degeneration of the latter. The trophozoites, measuring 0.3–5.0 mm in length by 0.3–1.2 mm in width, are readily visible with the unaided eye. They usually lie end-to-end, and in adjacent fibers, one upon another, forming concentrations up to 6–7 cm in diameter. In the great majority of the infected fish the concentrations were close to the vertebral column, in many instances completely surrounding the vertebrae in the region of the infection. The remainder of the concentrations occurred just beneath the skin, mainly near the dorsal and ventral margins of the fish. In most of the parasitized fish the concentrations were anterior to the middle of the body, the largest and oldest being adjacent to the body cavity. All concentrations of trophozoites have a papillae-like appearance. Some of the trophozoites are brownish (usually those in the center of a concentration), while others are whitish (generally at the periphery). No visible morphological difference was noted between these differently pigmented trophozoites. Occasionally, concentrations were observed in which the trophozoites had been partially or completely calcified, forming a hard crust. Apparently, this was due to a host reaction to the presence of the parasite in its tissue. Many fish were observed in which individual trophozoites were scattered throughout or in parts of the flesh, and were not concentrated.

Microscopic examination of several trophozoites revealed that they contained many minute, thick-walled cysts, each of which further contained many egg-shaped spores massed together. The cysts measured 0.011–0.023 mm in length by 0.010–0.019 mm in width, while the individual spores measured 0.004–0.007 mm in length by 0.002–0.004 mm in width. No attempt was made to determine the morphology of the spores.

Examination of 2,032 fillets (representing 1,016 fish) from the area below Cape Cod, extending from Block Island to No Man's Land, revealed that 138 (6.8 per cent) were infected. In the

three samples from this area the per cent of infection varied from 3.9–10.5 per cent. Of the 2,074 fillets (representing 1,037 fish) examined from Cape Cod Bay, 142 (6.8 per cent) were infected. The per cent of infection in the six samples from this area varied from 1.0–34.0 per cent. A small sample of 46 fillets (23 fish) from Casco Bay, Maine contained only one (2.2 per cent) infection. It is significant to note, from the above data covering the examination of 4,152 eelpout fillets during the period from March 24 to August 18, 1943, that some infections were found regardless of the fishing grounds on which the fish were caught.

It was deemed necessary, as an aid in detecting and culling out infected eelpout fillets, to determine the relative effectiveness of various methods of candling these fillets. The candling apparatus employed was the same as that used for rosefish (*Sebastes marinus*) in detecting the presence of, or lesions caused by, the parasitic copepod, *Sphyrion lumpi*. The apparatus consisted essentially of a square or rectangular wooden frame, a lightly frosted plate glass resting on the frame, and two or more electric light bulbs beneath the glass. In candling eelpout fillets commercially, they are pulled rapidly across the surface of the frosted glass, the lights beneath usually exposing in the flesh some or all of the concentrations of trophozoites and scattered individuals. The results of various methods of candling the same lot of eelpout fillets are shown in Table 1. By the rapid candling of both sides of the skinned fillets all concentrations of trophozoites plus some scattered individuals were detected (21 per cent infection). The detailed candling of both sides of the same fillets revealed additional infections (13 per cent more) due solely to the presence of individual trophozoites scattered throughout or in parts of the fillets. All per cents of infection obtained by the methods employed are compared to that of the last method, the latter showing the total per cent of fillets infected.—Jacob H. Fischthal, *U. S. Fish and Wildlife Service, Cambridge, Mass.*

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No. 4, August 10.
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No. 6, December 10.